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Using EIA to screen *Capsicum* spp. germplasm for capsaicinoid content

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Abstract

An enzyme immunoassay was evaluated for its ability to quantify total capsaicinoids in fruit of 16 genotypes representing four species of *Capsicum*, in comparison with high-performance liquid chromatography (HPLC). Concentrations of capsaicinoids in samples ranged from 5.1 to 4284 ppm, and from 0.6 to 3467 ppm, as determined by enzyme immunoassay (EIA) and HPLC, respectively. Lowest concentrations of capsaicinoids occurred in sweet bell and pimento types (*C. annuum* L.), and the highest concentration occurred in a small-fruited 'bird'-type accession of *C. frutescens* L. (PI 593924). Estimates of capsaicinoids obtained by EIA and HPLC were highly correlated ($R^2 = 0.996$). Data suggest that EIA is an effective means for estimating total capsaicinoids in extracts of fresh chile fruits.
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1. Introduction

Pungency is a major quality-determining factor in chile (Zewdie & Bosland, 2000a). As such, the availability of data on pungency is frequently an important criteria for selection of genotypes from a genebank for use in crop improvement or other research-related or commercial activities. A wide range of pungency values are known to occur in the *Capsicum* spp. genepool (DeWitt & Bosland, 1993). However, data on pungency among the accessions in *Capsicum* genebanks are

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currently limited due in part to the expense, technical expertise and equipment required for its analysis using the currently accepted standard analytical technique, that is, high-performance liquid chromatography (HPLC) (Wall & Bosland, 1998).

The degree of pungency in *Capsicum* fruit is proportional to the combined concentrations of the various vanillyl amides that are collectively referred to as capsaicinoids (Suzuki & Iwai, 1984). Capsaicin (CAP) and dihydrocapsaicin (DHC) are generally the most prevalent capsaicinoids in chiles and frequently account for more than 95% of the total capsaicinoids present (Bennett & Kirby, 1968; Mathus, Dangi, Dass, & Malhotra, 2000). Although typically present in very low concentrations, numerous analogues of CAP, which vary in their relative pungencies (Perkins et al., 2002), have been reported (Suzuki & Iwai, 1984; Krajewska & Powers, 1988; Constant & Cordell, 1996; Kobata, Todo, Yazawa, Iwai, & Watanabe, 1998). The relative concentrations of these analogues vary with taxa and genotype (Zewdie & Bosland, 2001).

Plant germplasm evaluation typically requires the analysis of a large number of samples, on an infrequent basis. This situation often presents germplasm curators or crop improvement specialists with two choices: (1) outsource the analysis or (2) invest in the equipment, supplies and technical training or support required to conduct the analysis in-house. The cost of outsourcing the HPLC analysis of capsaicinoids in hundreds of samples could be prohibitive for some programs, as could be the purchase of specialized equipment for a limited use or one-time application. Enzyme immunoassay (EIA) offers several potential advantages when compared to HPLC for routine screening of total capsaicinoids in fresh fruit of *Capsicum* spp. These include a reduced investment required in the equipment, supplies, and training of personnel required to conduct the assay. These variables have been noted previously as justifications for the development of alternative assays for capsaicinoids (Wall & Bosland, 1998).

Our objective in this study was to evaluate an EIA originally developed for quantification of total capsaicinoids in salsa (Perkins et al., 2002) as a means to quantify total capsaicinoids in fresh fruits of *Capsicum* spp., and to briefly review factors that can effect efforts to accurately estimate CAP content.

2. Materials and methods

Fruits for analysis were harvested at maturity from plants grown on the Georgia Experiment Station, Griffin, GA. Genotypes analyzed and their geographic origin are presented in Table 1.

A minimum of 30 fully mature fruit per genotype were harvested, bulked, chopped into small ($<5\,mm^3$) pieces, and subsequently blended to a slurry using a commercial food processor. Five grams of the slurry were homogenized on ice in 25 ml of prechilled 100% MeOH for 3 min in a 50 ml centrifuge tube using a Tissumizer (Tekmar Corp., Cincinnati, OH) homogenizer at speed setting 8. The homogenate was centrifuged at 15 000*g* for 15 min at 20°C, and the supernatant containing total capsaicinoids was removed and stored in amber vials at −4°C until utilized.

EIA was conducted using the CAP Test Kit supplied by Beacon Analytical Systems (Presumpscot, ME) following the manufacturer's instructions. In as much as the effective range of the EIA assay was relatively narrow (0.1–2.0 ppm) and the known range for capsaicinoids in *Capsicum* fruit tissue is quite wide (0 to more than 5×10^5 ppm), serial dilutions from 1:10 to 1:1000 were prepared with 10% MeOH (the minimum concentration required to maintain the

Table 1
Capsicum germplasm analyzed for capsaicinoid content by EIA and HPLC

ID no.	Species	Country of origin	Status[a]	Name	Capsaicinoids (ppm)	
					EIA	HPLC
Grif 974	*C. Annuum*	China	Cultivated		5.1	1.1
Grif 1570	*C. Annuum*	Yemen	Cultivated		169	123
Grif 12453	*C. Annuum*	Albania	Cultivated	Kosova	34	17.1
Grif 14090	*C. Annuum*	Guatemala	Landrace		638	523
Grif 14094	*C. Annuum*	Guatemala	Landrace		495	356
Grif 14221	*C. Annuum*	Paraguay	Cultivated		13	3.1
Grif 14379	*C. Annuum*	India	Unknown		2699	2418
PI 298647	*C. Annuum*	Spain	Cultivated	Pimento bola	6.5	0.6
PI 370375	*C. Annuum*	Yugoslavia	Cultivated	Dolga blaga	14	10.3
Grif 14142	*C. Baccatum*	Paraguay	Landrace		774	760
PI 590506	*C. Baccatum*	Bolivia	Landrace		150	113
PI 596055	*C. Baccatum*	Bolivia	Landrace		167	115
PI 593926	*C. Chinense*	Ecuador	Landrace		819	703
Grif 14084	*C. Frutescens*	Guatemala	Landrace	Diente de perro	3581	3160
Grif 14088	*C. Frutescens*	Guatemala	Cultivated	Chile blanco	558	510
PI 593924	*C. Frutescens*	Ecuador	Landrace		4284	3467

[a] International Plant Genetic Resources Institute (1995). Descriptors for *Capsicum*. Rome, Italy.

capsaicinoids in solution) from each original extract. Replicate control samples of 0.0, 0.1, 1.0 and 2.0 ppm CAP in 10% MeOH, included with the assay kit, were run separately and were also included in all assays. Sample extracts (100 μl) were pipetted into wells of a 96-well plate. Enzyme conjugate (100 μl) was added to each sample and mixed by pipetting up and down several times. Enzyme conjugate–sample mixtures (100 μl) were transferred to antibody-coated reaction wells and allowed to incubate for 10 min at room temperature. Plates were washed four times with tap water. Substrate (100 μl) was then added to each well, and the plates incubated for an additional 10 min at room temperature. Stop solution (100 μl) was then added to each well and reactions were read at 405 and 620 nm on a Molecular Devices (model Emax) microplate reader. Extracts were also analyzed for capsaicinoid (CAP and DHC) content by HPLC with fluorescent detection as described by Perkins et al. (2002).

3. Results and discussion

Preliminary studies conducted using the control CAP solutions included with the EIA kit examined the linearity and the reproducibility of the assay. Under these conditions, estimates were highly reproducible (mean of 6 replications) with a coefficient of variation (CV) of $<0.05\%$, and linear ($R^2 > 0.999$). Correlation of control sample (0, 0.1, 1.0 and 2.0 ppm) values as determined by EIA and HPLC was also high ($R^2 > 0.99$). When samples of accession numbers PI 590506, PI 593926 and Grif 14379, selected as representative examples of the genotypes to be analyzed, were utilized in a subsequent study, the CV (mean of 6 replicate assays/genotype) was $<0.1\%$.

Capsaicinoid values among all samples analyzed ranged from 5.1 to over 4000 ppm, and from 0.6 to 3467 ppm, as determined by EIA and HPLC, respectively (Table 1). The lowest concentrations were observed in *C. annuum* bell-type sweet pepper Grif 974, and pimento-type PI 298647. The highest concentration was detected in a small-fruited 'bird' pepper (*C. frutescens*, PI 593924). Capsaicinoid concentration did not appear to be correlated with specific taxa as the distribution of capsaicinoid values from fruit of *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens,* overlapped. Zewdie and Bosland (2001) demonstrated that both total capsaicinoid content and the concentrations of individual capsaicinoids are not unique to a particular species, although both can be affected by genotype.

The correlation between capsaicinoid estimates obtained from EIA and HPLC across all samples was high ($R^2 > 0.99$, Fig. 1) indicative of the robust nature of the EIA assay in comparison with HPLC. This is a similar value to that reported by Perkins et al. (2002). Capsaicinoid values as determined by EIA were consistently higher than those obtained following analysis of identical extracts by HPLC. A similar trend was also observed by Perkins et al. (2002). We attribute these higher estimates to the fact that the HPLC assay quantified only CAP and DHC, and not other naturally occurring homologs. In contrast, due to cross-reactivity of the antisera, values for capsaicinoids as determined by EIA included nordihydrocapsaicin (Perkins et al., 2002) in addition to CAP and DHC, and possibly other homologs closely related to these. Differences in estimates of capsaicinoids as determined by EIA and HPLC were most evident at the low end of the pungency range (e.g., PI 298647, Grif 14221 and Grif 974). We attribute a portion of this variability to the nonspecific interference (matrix effect) present in these minimally diluted samples, but cannot totally discount the potential for a matrix effect at higher dilutions.

Pungency is a quantitatively inherited characteristic (Zewdie and Bosland, 2000a). Pungency within genotypes has been shown to vary dramatically as a result of various physiological and environmental factors including, but not limited to, position of the fruit on the plant (Zewdie & Bosland, 2000b), stage of maturity (Iwai, Suzuki, & Fujiwake, 1979; Contreras-Padilla & Yahia, 1998), light (Iwai, Lee, & Kobashi, 1977), fertilization (Osman & George, 1984), and the general

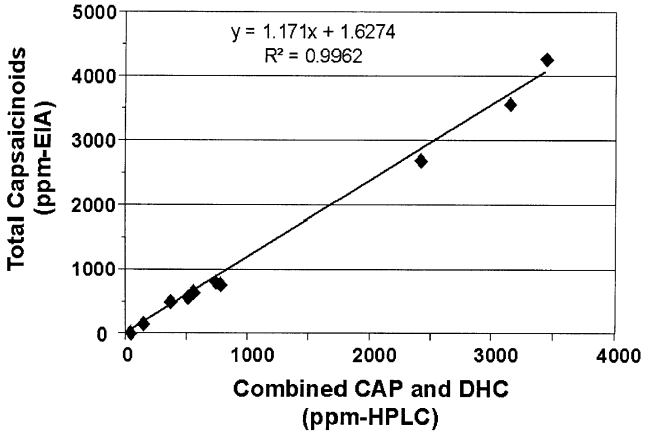

Fig. 1. Correlation of capsaicinoid concentration in fruit extracts of 16 *Capsicum* genotypes as determined by EIA and HPLC.

culture environment (Zewdie & Bosland, 2000c). Harvell and Bosland (1997) suggested that environmental effects on pungency could have a greater effect than genotype. Thus, any value for capsaicinoid content that is assigned to a particular genotype might best be thought of as an approximation, and valid only under certain well-defined environmental and physiological conditions. Given the large number of variables influencing pungency, it would seem desirable that for purposes of increasing the accuracy of germplasm evaluation, that all genotypes to be analyzed for CAP or total capsaicinoids be grown simultaneously and in a single location, to the extent possible. Since this is often impractical or impossible, an alternative means to facilitate accurate comparisons of future estimates of capsicinoids obtained over periods of time or in different locations would be the inclusion of a set of reference genotypes in each analysis/round of evaluation, in conjuction with the use of well-defined sampling techniques.

4. Conclusion

While capsaicinoid values as determined by enzyme immunoassay (EIA) differed from those obtained using high-performance liquid chromatography (HPLC), these differences were generally small. EIA provides an alternative means to estimate total capsaicinoid content in fresh chile fruit. We suggest that this technique is appropriate for use in *Capsicum* germplasm evaluation and improvement programs and in other instances where periodic and routine analysis of pungency is required. The EIA analysis as described is not suitable for the quantification of individual capsaicinoids, or for generating capsaicinoid profiles. In these instances, HPLC would be a preferred technique.

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